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Note

Studies on the interactions between glycosylated β^3-peptides and the lectin *Vicia villosa* by saturation transfer difference NMR spectroscopy

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ABSTRACT

Saturation transfer difference (STD) NMR spectroscopy was used to study the interaction of the lectin *Vicia villosa* ($VVLB_4$) with α-D-GalNAc glycosylated β^3-peptides. The data were compared to those obtained with the monosaccharides D-Gal, D-GalNAc, and D-Glc as well as with those obtained with the Tn antigen α-glycopeptide (D-GalNAc-α-*O*-Ser/Thr), molecule naturally recognized by *V. villosa*. Evidence that the lectin also recognizes glycosylated β^3-peptides and has close contact with both the sugar and amino acid moieties was obtained.

The recognition of glycoconjugates is an important process in biological systems and is associated with autoimmune- and infectious diseases, and is frequently in the form of carbohydrate–protein interactions.[1] Carbohydrates help to stabilize the conformation of glycoproteins. The understanding of how they are recognized by the binding sites of proteins, antibodies, and enzymes is a topic of major carbohydrate interest. *N*-Acetyl-α-D-galactosamine (α-D-GalNAc), O-linked to the protein domain is an important constituent of the carbohydrate moieties of glycoproteins.[2]

Lectins are multivalent carbohydrate-binding proteins of non-immune origin which display a wide diversity of sugar binding specificity in different biological systems. They often served as structural models for the analysis of protein–carbohydrate interactions, and have received a considerable attention as biological tools to detect subtle variations in carbohydrate structures.[3] One of the most important lectins for detecting exposed Tn determinants (D-GalNAc-α-*O*-Ser/Thr) on cancer cells is the isolectin B_4 from *Vicia villosa* ($VVLB_4$)[4,5] which belongs to the family with overall specificity for GalNAc/Gal.[6,7] The acetamido moiety of α-D-GalNAc is often a dominant or significant recognition determinant.[8] $VVLB_4$ has been used to investigate the differences in Tn antigen expression in many carcinogenic tumors including breast, prostate, lung, and pancreatic cancers.[9,10] A level of expression in the Tn determinants often correlates well with carcinoma differentiation and aggressiveness.[11]

Foldamers are unnatural polymeric molecules capable of adopting well-defined secondary structures.[12] They offer a broad pallet of building blocks for the construction of molecules helpful in understanding protein folding and function.[13] β-Peptides which are closely related to the ubiquitous α-peptides are one of the most intensively studied foldamers.[14] They differ from α-peptides by an additional methylene group present in the peptide backbone. It was shown that β^3-peptides, despite this additional methylene group have a high propensity to form 3_{14}-helices similar to those found in nature.[15] These compounds are interesting in regard of, for example, antibacterial and hemolytic or antiproliferative properties[16,17] and are stable toward proteolytic, and metabolic degradation.[18,19] The resulting hybrid conjugates such as glycosylated β^3-peptides are expected to be of interest to study the effects of glycosylation on the backbone structure, and also to investigate how the unnatural backbones affect the properties and the biomolecular recognition of the attached natural sugars.

It has been shown recently using surface plasmon biosensor technology that the lectin *V. villosa* binds to glycosylated β^3-peptides.[20] Herein we have studied the interaction between $VVLB_4$

Abbreviations: STD NMR, saturation transfer difference NMR; $VVLB_4$, lectin from *Vicia villosa* (isolectin B_4); α-D-GalNAc, *N*-acetyl-α-D-galactosamine; Gal, galactose; Glc, glucose.

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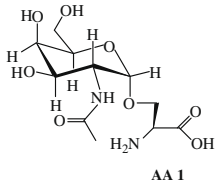
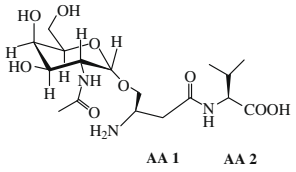
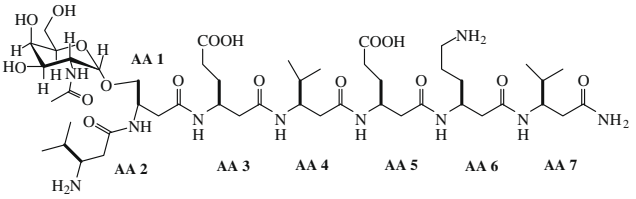


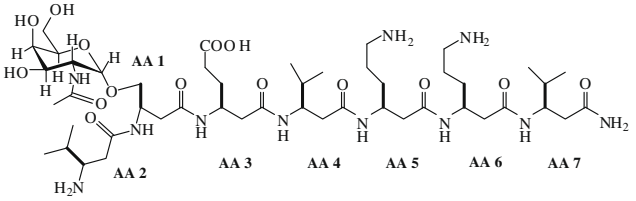
1
(Tn antigen)
Ser[1](αGalNAc)



2
β[3]hSer[1](αGalNAc)-Val[2]



3
β[3]hVal[2]-β[3]hSer[1](αGalNAc)-β[3]hGlu[3]-β[3]hVal[4]-β[3]hGlu[5]-β[3]hOrn[6]-β[3]hVal[7]-NH$_2$



4
β[3]hVal[2]-β[3]hSer[1](αGalNAc)-β[3]hGlu[3]-β[3]hVal[4]-β[3]hOrn[5]-β[3]hOrn[6]-β[3]hVal[7]-NH$_2$

Scheme 1. Structure of the Tn antigen (**1**) and glycosylated β[3]-peptides (**2**–**4**).

and glycosylated β[3]-dipeptide and β[3]-heptapeptides linked to α-D-GalNAc residue using STD NMR spectroscopy.[21,22] The aim of this work was to identify binding, the parts of the ligand that are in contact with the lectin and to determine the differences between binding of the natural α-glycopeptide, the Tn antigen and unnatural oligomeric β[3]-glycopeptides.

^{1}H and ^{13}C NMR signals for the Tn antigen (**1**) and glycosylated β[3]-peptides (**2**–**4**) (Scheme 1) were assigned from COSY, TOCSY, NOESY, and HSQC-DEPT experiments (Table 1). The interaction of the monosaccharides, D-GalNAc, D-Gal, D-Glc with VVLB$_4$ was also investigated. With D-Glc, there were no signals in the STD NMR spectra suggesting that this compound does not bind to the lectin. STD effects were observed for D-GalNAc and D-Gal (Fig. 1) indicating binding. Largest effects were seen for the H-3 and H-4 signals of α-anomer showing than the corresponding protons have the closest contact to the protein. It also shows how the lectin discriminates between α-D-Gal and β-D-Gal. STD effects were also observed for the CH$_3$, H-3, and H-4 signals of D-GalNAc in the alfa-configuration (Fig. 1), but for the same concentrations of ligand and protein the STD effects were much weaker than with D-Gal. To determine whether this was due to different types of binding or to tighter binding competition STD NMR experiments were performed.[23–25] In these experiments, the STD signals of the low-affinity ligand are monitored while adding another ligand. If this new ligand interacts with the same binding site as the low affinity ligand and with a higher affinity, a decrease in intensity or disappearance of the STD signals of the lower affinity ligand is observed. Thus, the addition of equimolar amount of D-GalNAc resulted in a large decrease in the STD signals of D-Gal, demonstrating that both compounds compete for the same binding sites and confirming that D-GalNAc exhibits higher affinity than D-Gal.

With the Tn antigen the saturation transfer was observed for the proton signals of the *N*-acetyl-D-galactosamine residue demonstrating tight contact with the protein. Signals from the serine residue were not observed (Fig. 2, compound **1**). These results are in good agreement with those obtained for the crystal structure of

Table 1
^{1}H and ^{13}C chemical shifts (ppm) at 283 K of Tn antigen (**1**) and glycosylated β[3]-peptides (**2**–**4**)

Compound	Residue	H-1/C-1, Hα/Cα	H-2/C-2, Hβ/Cβ	H-3/C-3, Hγ/Cγ	H-4/C-4, Hδ/Cδ	H-5/C-5, Hε/Cε	H-6,6′/C-6	NAc
1	α-GalNAc	nd[a]	3.91	3.76	3.87	3.85	3.63	1.95
			53.9	67.6	68.1	68.5	61.1	21.8
	α-Ser[1]	4.13	3.64, 3.94					
		49.3	66.5					
2	α-GalNAc	4.86	4.15	3.88	3.89	3.83	3.66	1.97
		97.4	49.3	67.2	68.0	70.9	60.9	21.5
	β[3]-hSer[1]	2.72	3.82	3.45, 3.86				
		33.7	47.7	66.3				
	α-Val[2]	4.05	2.11	0.85	0.88			
		58.8	29.4	16.2	18.2			
3	α-GalNAc	4.81	4.07	3.83	3.88	3.80	3.65	1.95
		96.8	49.2	67.1	67.9	70.7	60.5	21.5
	β[3]-hSer[1]	2.34, 2.55	4.37	3.51, 3.60				
		36.8	46.2	68.0				
	β[3]-hVal[2]	2.48, 2.61	3.35	1.87	0.90	0.90		
		36.4	53.2	29.5	16.5	16.5		
	β[3]-hGlu[3]	2.29, 2.37	4.08	1.53, 1.59	1.58, 1.77			
		40.4	46.1	22.85	28.67			
	β[3]-hVal[4]	2.22, 2.48	3.97	1.68	0.80	0.80		
		37.1	51.6	31.3	17.6	17.6		
	β[3]-hGlu[5]	2.29, 2.37	4.08	1.53, 1.59	1.58, 1.77			
		40.4	46.1	22.9	28.7			
	β[3]-hOrn[6]	2.27, 2.36	4.08	1.40, 1.50	2.28	2.88		
		40.8	46.1	30.04	30.0	38.5		
	β[3]-hVal[7]	2.22, 2.48	3.97	1.68	0.79	0.79		
		37.3	51.6	31.3	16.5	16.5		